

# Analysis of mixed linkage $\beta$ -glucan content and structure in different wheat flour milling fractions

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## ABSTRACT

$\beta$ -glucan is a dietary fibre component with health benefits that relate to its structure and solubility. The polysaccharide structure consists predominantly of  $\beta$ -(1–4) linked cellotriosyl (G3) and cellotetraosyl (G4) units joined together with  $\beta$ -(1,3) linkages. The ratio of G3:G4 blocks affects the solubility with very high or very low ratios causing lower solubility. Wheat, a major staple crop, is a source of  $\beta$ -glucan in the human diet; however, there is a lack of research on  $\beta$ -glucan in wheat, especially white flour which is used in many food products. Here we quantified  $\beta$ -glucan in different wheat milling fractions, showing a low content in the first and second break (white) flour fractions (0.2%) with increasing amounts in bran flour (0.5%), wholemeal (0.8%) and bran (2.8%). A high proportion (30%) of  $\beta$ -glucan in the white flour fractions was soluble, while in bran a far smaller proportion (10%) was soluble. In agreement with differences in solubility, the G3:G4 ratio also differed, with the white flour fractions having lower ratios (~2.5) and bran-containing fractions having higher ratios (~3.8). We conclude that while total  $\beta$ -glucan in white flour is low, it is substantially soluble, and that high extraction and wholemeal flours have the potential to be a significant source of  $\beta$ -glucan.

## 1. Introduction

Worldwide there are increases in chronic diseases, notably cardiovascular disease, type 2 diabetes and various types of cancer. This is associated with increased life expectancy and changes in lifestyle including the adoption of the “western diet” which is rich in refined foods (Deehan and Walter, 2016). Refined foods are depleted in dietary fibre, the intake of which has been associated with a reduced risk of several chronic diseases (Gill et al., 2021). Cereals are major sources of dietary fibre, providing about 40% of the total intake in the UK, half of which is provided by breads (Lockyer et al., 2016). Wheat, especially white wheat flour used in the production of bread and other foods (including Asian noodles) worldwide, is therefore a good target for enrichment in dietary fibre in order to improve health outcomes.

One of the most widely studied types of cereal dietary fibre is (1–3) (1–4)- $\beta$ -d-glucan ( $\beta$ -glucan), particularly in relation to reduced risk of cardiovascular disease and type 2 diabetes (Henrion et al., 2019). It is suggested that the health benefits associated with  $\beta$ -glucan relates to its physicochemical properties, particularly solubility and viscosity (ibid). It is probable that both soluble and insoluble  $\beta$ -glucan are associated with improvements in cardiovascular health and blood glucose

regulation, while insoluble  $\beta$ -glucan further benefits gut motility and faecal bulking (Gill et al., 2021).

The solubility of  $\beta$ -glucan is determined by its structure (Lazaridou and Biliaderis, 2007).  $\beta$ -Glucan consists of blocks of  $\beta$ -(1–4) linked D-glucopyranoside units, that are in turn linked to each other with  $\beta$ -(1–3) bonds. Most of these blocks comprise 3 (G3) or 4 (G4) glucose monomers, although higher degrees of polymerisation (DP) are also observed (Johansson et al., 2000). The distribution of G3 and G4 blocks is irregular, leading to an irregular structure overall. However, the lengths of the blocks of  $\beta$ -(1–4) linked units affect the physicochemical behaviour, such that the longer the block is, the greater the propensity of individual polymers to self-associate and form insoluble aggregates. Variation in the structure of  $\beta$ -glucan therefore may affect the health benefits and functional properties of  $\beta$ -glucan in foods.

A vast volume of work has been carried out on  $\beta$ -glucan fractions from oats and barley, where it forms a high proportion of total cell wall polysaccharides in the starchy endosperm and aleurone. Barley contains about 20% dietary fibre (Andersson et al., 2008), 75% of which is  $\beta$ -glucan in the starchy endosperm (Collins et al., 2010), whereas oat contains about 10% dietary fibre (Manthey et al., 1999; Jokinen et al., 2021) with 2.9–6.7%  $\beta$ -glucan content on a dry weight basis (Collins

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et al., 2010; Jokinen et al., 2021). In oats, 80–90% of the  $\beta$ -glucan is water-extractable (Collins et al., 2010), while in barley 50–70% is water-extractable (Åman and Graham, 1987; Lee et al., 1997). By contrast,  $\beta$ -glucan accounts for only 20–25% of the total cell wall polysaccharides in the starchy endosperm and aleurone of wheat and most studies have focused on whole grains. These studies have shown that it is only poorly extracted (i.e. soluble) in aqueous solvents (Beresford and Stone, 1983), possibly due to entrapment in arabinoxylan (AX), which is the predominant dietary fibre in wheat (Faulds and Williamson, 1995; Izydorczyk and MacGregor, 2000).  $\beta$ -Glucan is deposited in the endosperm cell walls earlier than AX during wheat grain development (at the cellularisation stage), while AX is detected later during endosperm differentiation (Philippe et al., 2006). The extraction of  $\beta$ -glucan is therefore increased under alkaline conditions that extract AX and/or digestion with xylanase (Ragaee et al., 2008) although alkaline treatment can also cause some depolymerisation (Beer et al., 1997).

Although  $\beta$ -glucan is a minor component in white wheat flour, about 0.5% on a dry weight basis, the high volume of consumption of breads, pasta, noodles and other products made with wheat makes it the major source of  $\beta$ -glucan in the diet of many populations, greatly exceeding the intakes from barley or oats (Supplementary Table S1). According to our calculations, Europeans consume the most  $\beta$ -glucan from wheat-based sources (estimated at 1.5 g/day per capita assuming 0.5%  $\beta$ -glucan content) which is half of the daily intake of 3 g recommended for health benefits (EFSA panel on Dietetic products Nutrition and Allergies, 2010; Henrion et al., 2019). One strategy to increase the  $\beta$ -glucan content of wheat-based foods is to supplement it with other flours (such as oat or barley). However, this will increase the cost of staple foods and the products may have lower consumer acceptability than more widely consumed white flour products. More attractive strategies are to increase the contents of  $\beta$ -glucan in conventional wheat-based products, either by increasing the content of  $\beta$ -glucan in white flour or by incorporating proportions of  $\beta$ -glucan-rich milling fractions. To underpin these approaches we have carried out detailed analyses of the content, structure and solubility of  $\beta$ -glucan in milling fraction of wheat, particularly white flour fractions which has been studied only to a limited extent by previous workers (Beresford and Stone, 1983).

## 2. Experimental

### 2.1. Flour samples

Milling fractions were prepared as described previously (Rodríguez-Ramiro et al., 2017). Whole grain from the UK hard breadmaking cultivar Hereward, grown at Rothamsted Research in 2014, was milled by Campden BRI using a Buhler-MLU-202 mill according to TES-CM-01. This resulted in 10 fractions (Supplementary Fig. S1), including six white flour fractions: three breaks (B1–B3) and three reductions (R1–R3). The remaining four fractions were bran overtails, bran flour, offal overtails and offal flour. The bran flour was obtained by passing the bran flakes collected from the break rolls through a bran finisher (essentially a beating/grinding and sieving process), producing a flour that is darker with a high percentage ash. The remnant of this step is the bran overtails (referred to simply as “bran” in this paper). A wholemeal flour was produced by recombining the 10 flour fractions as described (Rodríguez-Ramiro et al., 2017). The milled fractions were stored at  $-20\text{ }^{\circ}\text{C}$  until use.

### 2.2. Quantification

Quantification was performed using the mixed linkage  $\beta$ -glucan kit from Megazyme (Megazyme, Bray, Ireland) according to the manufacturer's instructions with some adjustments. The amount of sample used depended on the milling fraction: for break 1, break 2, wholemeal and bran flour 190 mg sample was used. For bran 160 mg was used. To wet

the samples, 0.4 mL aqueous ethanol (50% v/v) was added after which 3.8 mL 20 mM sodium phosphate buffer (pH 6.5) was added before incubation in a boiling water bath. All other steps were as described in the instruction manual provided in the kit. The effect of xylanase was investigated by adding endo-1,4- $\beta$ -xylanase (GH11, recombinant from *Neocallimastix patriciarum*) (Prozomix, Haltwhistle, UK) at a concentration of 2U/mL reaction volume during the lichenase digestion step. A control sample (barley) of known  $\beta$ -glucan content was analysed alongside test samples.

### 2.3. Separating soluble and insoluble fractions

Samples were incubated in 1 mL water (prior to fingerprinting) or 3.8 mL 20 mM sodium phosphate buffer (pH 6.5) (prior to quantification) for 5 min at  $50\text{ }^{\circ}\text{C}$  and 750 rpm on a Thermomixer (Mixer HC, Starlab, UK) before centrifugation at  $1000\times g$  for 10 min. The supernatant was transferred to a separate tube for the analysis of soluble  $\beta$ -glucan and the pellet was resuspended in water (for fingerprinting) or 20 mM sodium phosphate buffer (pH 6.5) (for quantification) to the original volume for the analysis of insoluble  $\beta$ -glucan. Initial extraction of soluble  $\beta$ -glucan was attempted at  $100\text{ }^{\circ}\text{C}$  instead of  $50\text{ }^{\circ}\text{C}$  as described above, but results had an unacceptably large coefficient of variation for both content and structural analysis, particularly in white flour fractions (Supplementary Tables S2 and S3), and so were not included in this study.

### 2.4. Fingerprinting

Fingerprinting to determine G3:G4 ratio was performed based on Ordaz-Ortiz et al. (2005) with modifications. Before fingerprinting, samples were treated with fructanase to remove fructan which caused interfering peaks especially at the G3 position in soluble fractions (Fig. S1). Samples (100 mg flour or prepared soluble/insoluble samples) were incubated in 1 mL of ultrapure water containing 10  $\mu\text{L}$  recombinant fructanase (GH32, *Aspergillus niger*) (Megazyme, Bray, Ireland) representing 20U exo-Inulinase and 1U endo-Inulinase activity. Incubation was performed at  $50\text{ }^{\circ}\text{C}$  for 45min and 750 rpm in a Thermomixer (Mixer HC, Starlab, UK), before drying under vacuum in a centrifuge concentrator (Concentrator plus, Eppendorf, UK) at  $45\text{ }^{\circ}\text{C}$  for approximately 4 h. Drying was stopped when samples were still moist to avoid over-dessication which hampered subsequent resuspension. After fructanase treatment all samples were resuspended in 1 mL of ethanol (80%) before being boiled in a water bath for 10 min. Samples were centrifuged at  $14.1\text{ k}\times g$  for 5 min before being washed in 1 mL 80% ethanol and then 1 mL 95% ethanol. After the final step samples were dried under vacuum in a centrifuge concentrator (as before) at  $45\text{ }^{\circ}\text{C}$  until completely dry. Fructanase-treated samples were then resuspended in a reaction mixture consisting of water containing 2U/mL of lichenase (GH26, native, from *Bacillus subtilis*) (Megazyme, Bray, Ireland) with or without the addition of 2U/mL endo-1,4- $\beta$ -xylanase (GH11, recombinant from *Neocallimastix patriciarum*) (Prozomix, Haltwhistle, UK), and incubated at  $40\text{ }^{\circ}\text{C}$  for 16 h 750 rpm. The reaction volume for total flour samples or insoluble fractions was 1 mL, and for soluble fraction it was 0.5 mL. This compensated for the small amount of  $\beta$ -glucan present in the soluble fraction compared to the insoluble fraction. Reactions were stopped by boiling in a water bath for 30 min, after which samples were centrifuged for 5 min at  $14.1\text{ k}\times g$  and supernatants filtered through a syringe-driven  $0.45\mu\text{m}$  PVDF filter (Whatman, UK). Samples were diluted 1:20 in 7  $\mu\text{M}$  D (+) melibiose ( $\geq 99\%$ , Sigma-Aldrich, UK) and analysed using HPAEC-PAD.

### 2.5. HPAEC-PAD

Carbohydrate fragments were separated on a CarboPac PA1 column (ThermoScientific, Hemel Hempstead, UK) using a Dionex ICS-6000 instrument (ThermoScientific, Hemel Hempstead, UK). Gradient

elution was used as described before (Ordaz-Ortiz et al., 2005). Fragments were detected using pulsed amperometric detection (PAD). Assignment of peaks were made by comparison with peak profiles of 3<sup>1</sup>-β-D-Cellobiosyl-glucose (G3) and 3<sup>1</sup>-β-D-Celotriosyl-glucose (G4) standards (Megazyme, Bray, Ireland). Peak analysis was performed using Chromeleon 7 Chromatography Data System (v7, Thermo-Scientific, Hemel Hempstead, UK). All peaks were visually inspected and manually integrated as required. The normalized weight % of G3 and G4 was calculated by normalizing to an internal standard (7 μM melibiose) and calculating the percentage of G3 and G4 where 100% is the sum of G3 and G4. The molar ratio of G3:G4 was then calculated based on the molecular weight of G3 (504.4) and G4 (666.6).

## 2.6. Derivatisation of oligosaccharides with 8-aminonaphthalene-1,3,6-trisulfonic acid (ANTS)

Samples were prepared as described for total flour fractions in *Fingerprinting* but without fructanase treatment or filtering. Labelling with ANTS was performed according to Kosik et al. (2012). Samples (50 μL) or standards (5 μL each of 1 mM stock solutions) were dried under vacuum in a centrifuge concentrator (Concentrator plus, Eppendorf, UK) at 60 °C until completely dry. Reaction mixtures were prepared by adding 10 μL DMSO buffer (50% DMSO v/v, 7.5% acetic acid v/v), 5 μL 0.2 M ANTS (prepared in 1.5% acetic acid v/v), and 5 μL 1 M NaCNBH<sub>3</sub> (prepared in DMSO) to dried samples. Reaction solutions were incubated at 37 °C for 16 h, after which they were dried under vacuum in a centrifuge concentrator (as before) at 45 °C until completely dry. Derivatised samples were then suspended in 100 μL 3 M urea, and stored at -20 °C.

## 2.7. Polysaccharide Analysis using Carbohydrate Gel Electrophoresis (PACE)

Derivatised samples were separated using polyacrylamide gel electrophoresis as described by Kosik et al. (2012) using 20% and 12% polyacrylamide gels. Bran samples were diluted 8x in 3 M urea before electrophoresis. Five microliters of sample or standard were loaded per well, equivalent to 0.25 mg 1st break and 0.03 mg bran. Electrophoresis was carried out for 30 min at 200 V and then 105 min at 1000 V, after which gels were visualized under UV light on a UVP GelDoc-ItTS2 Imager (Ultra-Violet Products Ltd., Cambridge, UK) with a green fluorescent protein filter fitted. For band quantification, relative density was measured using ImageJ (1.51j8) (Schneider et al., 2012) and expressed per gram of flour applied to the gel. For comparison between gels, all bands of interest were normalized to a known standard (Xylose hexaacarbaride, 5 μL of a 1 mM stock).

## 2.8. Statistical analysis

All analyses were performed using R Statistical Software (v4.2.2). Unpaired, two-sided *t*-test was done using the *rstatix* R package (v0.7.1; <https://cran.r-project.org/package=rstatix>). Analysis of variance was done using the *stats* R package (R Core Team, 2022). Tukey's HSD test was done using the *agricolae* R package (v1.3.5; <https://cran.r-project.org/package=agricolae>).

## 3. Results

Wheat grains were milled on a Buhler laboratory mill to give 10 fractions: six white flours, bran, offal and flours recovered from bran and offal. The combined yield of the white flour fractions was 76.4% (Rodriguez-Ramiro et al., 2017), compared with commercial flour yields of about 78%. The 10 fractions were recombined in the proportions in which they were recovered to give wholemeal and four fractions selected for detailed analysis, namely break 1, break 2, bran flour and bran. Breaks 1 and 2 are the purest white flour fractions, corresponding

to the central starchy endosperm of the grain, and accounted for 12% and 7.8% of the total recovered fractions, respectively. The bran flour (1% total recovered fractions) is recovered by sieving the bran and comprises a mixture of starchy endosperm (which is likely to include the sub-aleurone cells) and bran while the bran fraction (9% total recovered fractions) consists almost entirely of the outer layers (mainly aleurone and pericarp) and embryo.

### 3.1. Quantification

The contents of total β-glucan in the five samples were initially determined using the conditions specified for the Megazyme total β-glucan kit. This showed significant differences between the β-glucan contents of all fractions, except breaks 1 and 2 (Fig. 1A). As expected, the bran fraction had the highest concentration of β-glucan (2.8 ± 0.2 % DW), followed by wholemeal (0.81 ± 0.12 %DW), bran flour (0.51 ± 0.03 %DW) and the first (0.21 ± 0.06 %DW) and second (0.18 ± 0.05 % DW) break flours.

In order to quantify the proportions of soluble and insoluble β-glucan, a comparison was carried out of the solubility at two temperatures, 100 °C and 50 °C. Although the amounts of β-glucan extracted from all fractions were greater at 100 °C, by 3–7%, the extracted fractions gave higher errors when subjected to structure analysis (discussed below; Supplementary Tables S2 and S3). A temperature of 50 °C was therefore used for quantification and further analysis.

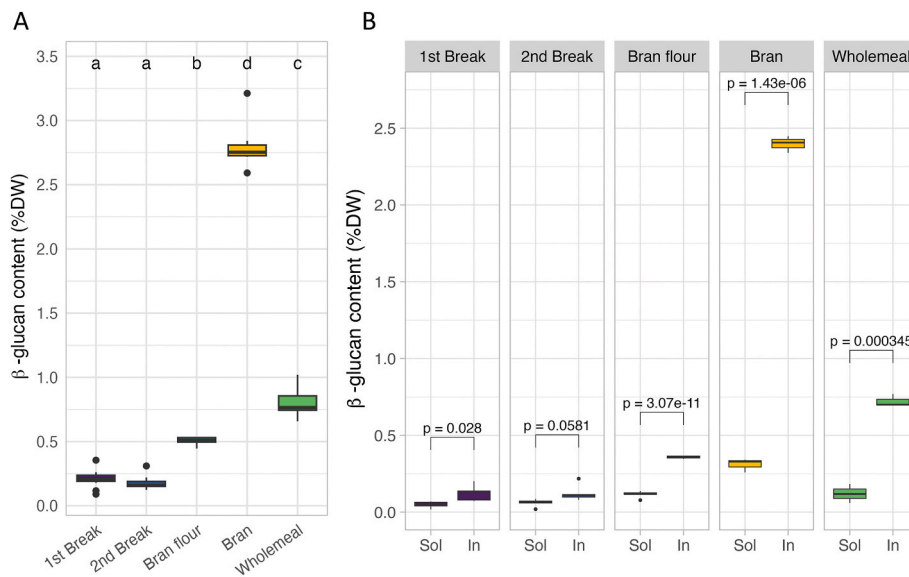
The proportions of β-glucan which were soluble and insoluble at 50 °C varied between the different fractions (Fig. 1B) with the first and second break fractions having the highest % of soluble β-glucan (28 and 33% soluble respectively) and the bran fraction the lowest proportion (11%). Bran flour had 25% soluble β-glucan and wholemeal had 14%.

It has been suggested that extractability of β-glucan may be reduced by the embedding of the polymer in a matrix of arabinoxylan polymers. The determination of total β-glucan was therefore carried out with the addition of endoxylanase (GH11) to digest arabinoxylan. This did not significantly affect the measured contents of β-glucan in the two white flour samples (Fig. 2). Although the mean amounts of β-glucan extracted from wholemeal and bran were slightly higher with the addition of endoxylanase (about 0.86% compared with 0.81% and 3.0% compared with 2.8%, respectively), these differences were not statistically significant (*p* = 0.413 and 0.699, respectively). Furthermore, the addition of xylanase also did not significantly affect the proportions of soluble and insoluble β-glucan when measured as separate fractions in the 1st and 2nd break flours (Supplementary Fig. S2).

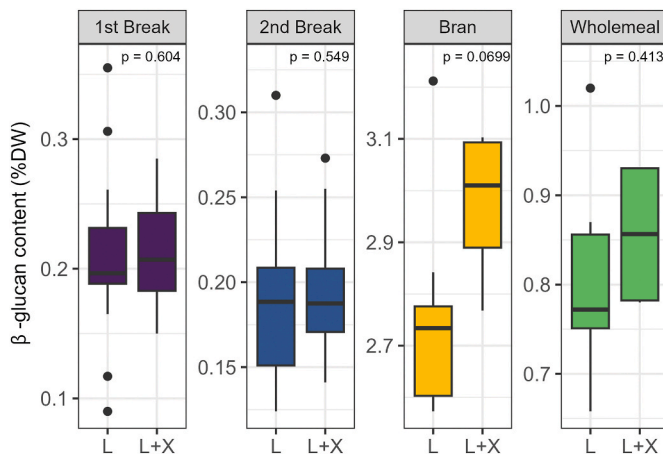
### 3.2. Structure

Differences in the structure of β-glucan in the fractions were determined by digesting the fractions with lichenase (β-glucanase) followed by separation and quantification of the gluco-oligosaccharide (GOS) fragments. Lichenase is a (1–3) (1–4)-β-D-glucan-4-glucanohydrolase (E. C. 3.2.1.73) which specifically cleaves the (1–4)-linkage of the 3-O-substituted glucose unit of β-glucan. It therefore releases mainly GOS of G3 and G4, although longer GOS are also released. The ratio of GOS released, and in particular the ratio of G3:G4 fragments, is therefore used as a "fingerprint" to compare β-glucan structure. Typical HPAEC-PAD separations of GOS from the 1st break and bran fractions are shown in Fig. 3.

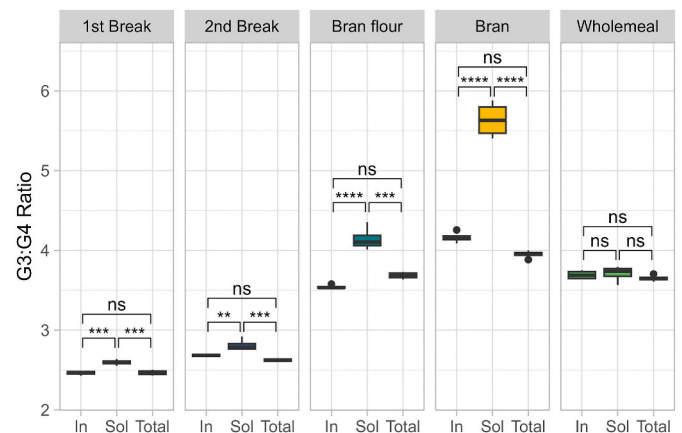
Determination of the ratio of G3:G4 GOS by HPAEC-PAD showed significant differences in the G3:G4 ratio between total β-glucan from the different fractions (Fig. 4 and Supplementary Fig. S3). The bran had the highest ratio at 3.95 ± 0.05, with the ratios for bran flour and wholemeal being slightly lower (3.69 ± 0.04 and 3.65 ± 0.04 respectively). A small but significant difference in G3:G4 ratio was also observed between the two white flour fractions with the second break being 2.63 ± 0.02 and the first break 2.47 ± 0.03. Fingerprinting of soluble and insoluble fractions revealed significant differences in the G3:



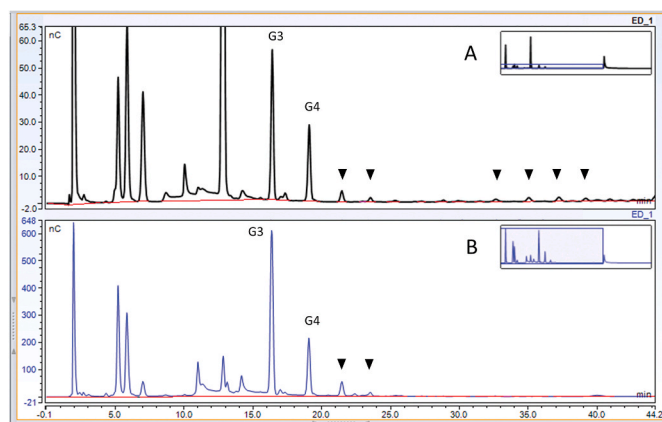
**Fig. 1.**  $\beta$ -Glucan content (% dry weight) of different milling fractions, calculated as total (A,  $n = 7-16$ ) and as soluble (Sol) and insoluble (In) fractions (B,  $n = 3-8$ ). Box plots show the mean and interquartile range. Outliers are indicated by dots. Letters indicate statistically significant differences at  $p < 0.05$ .



**Fig. 2.**  $\beta$ -Glucan content (% dry weight) in different milling fractions, calculated in whole samples treated with lichenase only (L) and lichenase and xylanase together (L + X). Box plots show the mean and interquartile range. Outliers are indicated by dots.  $N = 4-24$ .



**Fig. 4.** G3:G4 ratio measured in insoluble (In), soluble (Sol) and total  $\beta$ -glucan from different milling fractions. Boxplots show mean and interquartile range. Outliers are indicated by dots. Asterisks indicate significant differences based on ANOVA and post-hoc Tukey HSD analysis. \* =  $p < 0.05$ , \*\* =  $p < 0.01$ , \*\*\* =  $p < 0.001$ , \*\*\*\* =  $p < 0.0001$ , ns = not significant.  $N = 4$  for all analyses.



**Fig. 3.** Typical HPAEC-PAD chromatograms obtained from 1st break (A) and bran (B), showing the position of G3 and G4 peaks after lichenase digestion, as well as some higher DP peaks indicated with arrow heads ( $\blacktriangledown$ ).

G4 ratio for all samples except wholemeal, with the ratio being higher in the soluble fraction (Fig. 4). The same overall pattern is observed when comparing the G3:G4 ratios of milling fractions extracted with different methods (total, soluble and insoluble  $\beta$ -glucan), with the 1st and 2nd breaks having much lower ratios compared to bran flour, bran and wholemeal (regardless extraction method; Fig. 4 and Supplementary Fig. S3). In general, the G3:G4 ratio was significantly different between fractions within any given extraction method, except for the total fraction where there was no statistical difference between bran flour and wholemeal, and in the soluble fractions where there was no difference between 1st and 2nd break flours (Supplementary Fig. S3).

Analysis by HPAEC-PAD also showed the presence of GOS of DP greater than 4 (Fig. 3), but these are present in small amounts and not readily quantified. Digests of total  $\beta$ -glucan from the 1st break (white flour) and bran fractions were therefore analysed using Polysaccharide Analysis using Carbohydrate Gel Electrophoresis (PACE) (Fig. 5). Comparisons of digests with and without endoxylanase showed no differences in the ratio of G3:G4 GOS quantified using image analysis (not shown), but the presence of a range of arabinoxylan oligosaccharides

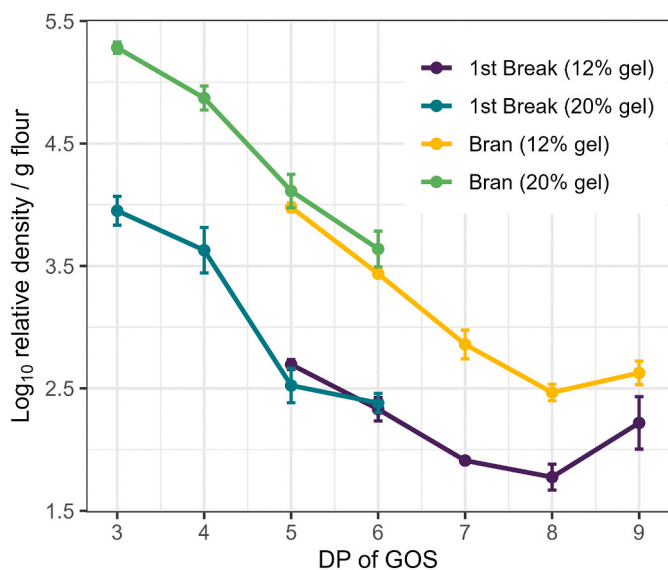


Fig. 5. Quantification of band densities representing different DP of  $\beta$ -glucan GOS as observed in PACE gels. Two different concentrations of gel were used to obtain resolution from DP3 to DP9. Mean density  $\pm$  SD is shown ( $n = 2-4$  per band).

(AXOS) in the digests with endoxylanase obscured the detection of GOS above DP4 (Supplementary Fig. S4A). Fractions digested with lichenase alone were therefore analysed by PACE using a lower concentration of acrylamide (Supplementary Fig. S4B) which resolved GOS with DP up to 9 in both flour and bran fractions. The GOS separated by PACE were quantified using image analysis, showing the presence of higher contents of all GOS in the bran extracts with decreasing proportions of GOS with increasing DP in both digests (Fig. 5). However, the proportion of DP9 was higher than that of DP8 in both digests and higher than that of DP7 in the flour digest.

#### 4. Discussion

We have shown that  $\beta$ -glucan represents about 0.2% of the dry weight of white wheat flour, of which about 30% is soluble in water at 50 °C. This is at the low end of the range reported for 49 white flour samples by Andersson et al. (1993), who reported means of 0.44% (range 0.25–0.63%) total  $\beta$ -glucan and 0.24% (range 0.19–0.41) soluble  $\beta$ -glucan. The low content in the present study may relate to the fractions used or to varietal or environmental factors, both of which can play a role (Manthey et al., 1999). The 1st break and 2nd break were selected as they represent the “purest” starchy endosperm, with minimum contamination with aleurone cells. This is demonstrated by their low contents of minerals (ash), which are concentrated in the aleurone layer and embryo and therefore used to monitor contamination of flour with bran. The 1st break and 2nd break fractions studied here contained 8.6 and 9.1 ppm iron, respectively, compared with 63.4 and 115.8 ppm in the bran flour and bran fractions, respectively (Rodríguez-Ramiro et al., 2017). The  $\beta$ -glucan contents determined here are also consistent with those reported previously (Bacic and Stone, 1980). Although the content of  $\beta$ -glucan was higher in bran than in white flour (about 3% compared to 0.2% dry wt.) this is still much lower than in “high- $\beta$ -glucan” cereals such as barley and oat (~5% dry wt.) (Andersson et al., 2008; Collins et al., 2010; Jokinen et al., 2021; Shewry et al., 2008). The difference in content and structure of  $\beta$ -glucan between milling fractions relates to the origin and function of the tissue from which it derives. The higher solubility of the  $\beta$ -glucan in the starchy endosperm cells may allow greater expansion during development and more rapid breakdown during germination. (Burton and Fincher, 2014). While we determined the distribution of  $\beta$ -glucan between different milling fractions of a single

variety (Hereward) in a single growing season, it is important to note that  $\beta$ -glucan content varies not only between species, but between cultivars, environment and with agronomic practices (Manthey et al., 1999). Therefore, while this study gives a general overview of distribution between milling fractions, there is scope for further study to identify differences in  $\beta$ -glucan between varieties and the effect of growing season or geography.

The extractability and solubility of  $\beta$ -glucan clearly depend on the conditions used, notably the temperature (Ragaee et al., 2008). We used 50 °C because the fraction extracted at this temperature gave more reproducible results when analysed by enzyme fingerprinting. Under these conditions a higher proportion of  $\beta$ -glucan was soluble in white flour, about 30% compared with only 11% in bran. The solubility of  $\beta$ -glucan is determined by its structure with the presence of long stretches of  $\beta$ -(1–4)-linked glucose residues resulting in insolubility due to hydrogen bond formation. This is demonstrated by the insolubility of cellulose, which is  $\beta$ -(1–4)-glucan. Extensive stretches of  $\beta$ -(1–4)-linked glucose residues in  $\beta$ -glucan are therefore often referred as “cellulose-like”. The relationship between the ratio of G3:G4 fragments released by lichenase treatment (which reflect the patterns of 1–3 : 1–4 bonds) and solubility is not clear. Although a G3:G4 ratio significantly different to 2.0 is considered to indicate lower solubility (Lazaridou and Biliaderis, 2007), the soluble fractions in bran flour and bran had very high G3:G4 ratios (4.14 and 5.64 respectively). Although the G3:G4 ratios in the soluble flour samples were lower compared to bran flour and bran ( $2.60 \pm 0.04$  for 1st break and  $2.81 \pm 0.07$  for 2nd break), all of the soluble fractions had higher G3:G4 ratios than the insoluble fractions. This contrasts with oats and barley where the opposite is observed (Johansson et al., 2000). Hence, it is clear that the G3:G4 ratio cannot be taken as an indicator of solubility and other factors must contribute, such as the chain length and proportions of larger fragments which could not be precisely quantified. It has been suggested that extractability of  $\beta$ -glucan may be reduced by the embedding of the polymer in a matrix of arabinoxylan polymers with phenolic cross-links and therefore increased by treatment with xylanase (Faulds and Williamson, 1995; Izidorczyk and MacGregor, 2000). We found no effect of endoxylanase treatment on the extraction of  $\beta$ -glucan from white flour and only small, and statistically not significant, effects on the extraction from wholemeal and bran. While we did not quantify the amount of arabinoxylan in these fractions it should be noted that the contents of arabinoxylan and  $\beta$ -glucan are both sensitive to environmental conditions, particularly heat stress, where higher temperatures tend to decrease  $\beta$ -glucan content and increase arabinoxylan content (Rakszegi et al., 2014).

Our results show that  $\beta$ -glucan is present at only low concentrations (about 0.2% dry wt.) in white flour of wheat, of which about 30% is extractable in water at 50 °C. It would be ideal to identify or develop a wheat variety that is high in  $\beta$ -glucan (particularly in the endosperm fraction) so that production of a high  $\beta$ -glucan wheat-based product would not require additional steps in the baking process to enrich it for this dietary fibre. Strategies to increase the  $\beta$ -glucan content of wheat bread include adding other flours with higher amounts, but this inevitably affects baking quality (Bucella et al., 2016). Therefore, these types of approaches require the input of stakeholders in the baking industry to ensure that products are commercially viable as well as attractive and affordable to consumers (bearing in mind that white bread is a staple food). It is clear that substantial increases in the content of  $\beta$ -glucan in white flour (by over two-fold) would be required to reach the recommended daily intake of 3 g (Supplementary Table S1). However, this does not mean that smaller increases would not have significant impacts on health outcomes as wheat is consumed as part of a mixed diet. By contrast, the content of  $\beta$ -glucan in whole grain is higher (0.8%  $\beta$ -glucan dry wt.) and only a small increase (to 1%) could allow consumers to reach their recommended intake (based on consumption levels in Europe, Supplementary Table S1). Furthermore, because  $\beta$ -glucan is concentrated in the aleurone, which represents only about 40% of the bran, this increase could probably be achieved by increasing the

extraction rate of the flour, reducing the impact on processing quality and consumer acceptability.

In conclusion, while the 1st and 2nd break flours had a higher soluble  $\beta$ -glucan content than the fractions containing bran (bran flour, bran and wholemeal), the contents in breaks 1 and 2 are low and products made with these flours would provide less than half of the recommended dietary intake of  $\beta$ -glucan. By contrast, wholemeal and high extraction flours have the potential to be significant sources of  $\beta$ -glucan.

#### Author statement

AL, PS - Conceptualization; Funding acquisition; Methodology; Writing - original draft; Writing - review & editing.

AP - Methodology; Investigation; Formal analysis; Visualization; Writing - original draft; Writing - review & editing.

All authors have seen and approved the final version of the manuscript. They warrant that the article is the authors' original work, hasn't received prior publication and isn't under consideration for publication elsewhere.

#### Declaration of competing interest

The authors have no conflicts of interest to disclose.

#### Data availability

Data will be made available on request.

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#### Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.jcs.2023.103753>.

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